

Physical properties of chondroitin sulphate/dermatan sulphate proteoglycans from bovine aorta

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Bovine aortic chondroitin sulphate/dermatan sulphate proteoglycans (PG-25, PG-35 and PG-50) were differentially precipitated with ethanol and analysed by a variety of chemical and physical techniques. The glycosaminoglycan chains of PG-25 and PG-35 contained a mixture of glucuronic acid and iduronic acid, whereas the uronic acid component of PG-50 was primarily glucuronic acid. In addition, various amounts of oligosaccharides containing small amounts of mannose, a galactose/hexosamine ratio of 1:1 and an absence of uronic acid were covalently linked to the core protein of all proteoglycans. The weight-average M_r (M_w) values of the proteoglycans determined by light-scattering in 4 M-guanidinium chloride were 1.3×10^6 (PG-25), 0.30×10^6 (PG-35) and 0.88×10^6 (PG-50). The s^0 values of the proteoglycans were distributed between 7 and 8 S, and the reduced viscosities, $\eta_{sp.}/c$, of all proteoglycans were dependent on the shear rate and polymer concentration. Electron microscopy of spread molecules revealed that PG-25 contained small structural units that appeared to self-associate into large aggregates, whereas PG-35 and PG-50 appeared mainly as monomers consisting of a core with various numbers of side projections. Hyaluronic acid–proteoglycan complexes occurred only with a small proportion of the molecules present in PG-35, and their formation could be inhibited by oligosaccharides. These results suggest the presence in the aorta of subspecies of chondroitin sulphate and dermatan sulphate proteoglycans, which show large variations in their physicochemical and inter- and intra-molecular association properties.

INTRODUCTION

The arterial wall contains a wide variety of proteoglycans (Engel, 1971; Wight, 1980; Berenson *et al.*, 1984). These macromolecules consist of a number of glycosaminoglycan chains covalently linked to a protein core. Biochemical studies of glycosaminoglycans from the aorta of different animal species demonstrate that these molecules are heterogeneous with respect to size, degree of sulphation and the nature of the uronic acid and hexosamine moieties (Sirek *et al.*, 1966; Kresse *et al.*, 1971; Hermelin *et al.*, 1976; Salisbury & Wagner, 1981; Radhakrishnamurthy *et al.*, 1982; Wight *et al.*, 1986). The structure and function of the core protein in aortic proteoglycans is less well understood. However, the variation in size and amino acid composition of the core proteins of proteoglycans from different tissues suggests that proteoglycans containing different glycosaminoglycan chains are separate gene products (Oegema *et al.*, 1979; Kapoor *et al.*, 1981; Swann *et al.*, 1983).

Until the present time there has been no detailed study of the physical properties of aortic proteoglycans. This is due perhaps to the exiguous amounts of this material in the tissue, and the difficulties involved in extracting it quantitatively in an undegraded form.

The present study was performed on a population of bovine aortic chondroitin sulphate/dermatan sulphate proteoglycans, which represented 60% of the total proteoglycans in this tissue. These proteoglycans were

extracted in 4 M-GdnHCl by non-shear-dependent techniques, and were subfractionated into IdoA- (iduronic acid)-rich (PG-25) and GlcA- (glucuronic acid)-rich (PG-35 and PG-50) proteoglycan fractions as previously described by Kapoor *et al.* (1981). The physical properties of these macromolecules were then determined over a wide concentration range by using light-scattering, viscometry, electron microscopy and ultracentrifugation techniques.

EXPERIMENTAL

Materials

GdnHCl, papain (2 × crystallized), trimethylchlorosilane, hexamethyldisilazane and various sugars used for g.l.c. were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). GdnHCl was purified by the method of Nozaki (1972) for use in all experiments. Dowex AG 2X-8 (400 mesh; Cl[−] form) was purchased from BDH Chemicals, Poole, Dorset, U.K. DE-23 DEAE-cellulose and DE-52 DEAE-cellulose were purchased from Whatman, Maidstone, Kent, U.K.

Glycosaminoglycans

Hyaluronic acid (rooster comb) and oligosaccharides (chain length C₁₀–C₁₄) prepared from hyaluronic acid were provided by Dr. J. Sheehan (University of Lancaster, Lancaster, U.K.).

Abbreviation used: GdnHCl, guanidinium chloride.

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Methods

The subfractionation of proteoglycans from bovine aorta into PG-25 (protein content 29%; IdoA/GlcA ratio 3:1), PG-35 (protein content 21%; IdoA/GlcA ratio 1:7) and PG-50 (protein content 12%; mainly consisting of GlcA residues alternating with hexosamine) was as described by Kapoor *et al.* (1981). Briefly, the procedure consisted of extracting minced aortic tissue with 10 vol. of 4 M-GdnHCl/50 mM-sodium acetate buffer (pH 5.8)/10 mM- Na_2EDTA /10 mM-benzamidine chloride/10 mM-6-aminohexanoic acid for 5 days at 4 °C. The extract was clarified by centrifugation in an MSE High Speed 18 centrifuge at 20000 *g* for 1 h at 4 °C. The supernatant was then concentrated 7-fold by ultrafiltration in an Amicon cell with a PM 10 filter, followed by dialysis at 4 °C against 6 M-urea/50 mM-Tris/HCl buffer, pH 6.5, containing the above proteinase inhibitors. The dialysis residue was then chromatographed on a DE-23 DEAE-cellulose column (50 mm \times 400 mm) equilibrated with the same buffer. The column was eluted with at least 4 bed volumes of (1) 6 M-urea, (2) 0.15 M-NaCl/6 M-urea and (3) 2 M-NaCl/6 M-urea, all in 50 mM-Tris/HCl buffer, pH 6.5. The last fraction (3) was further purified by DE-52 DEAE-cellulose chromatography in 6 M-urea with a linear gradient of 0.1–2 M-sodium acetate buffer, pH 5.8, at 4 °C. The main uronic acid peak was pooled and chromatographed on a Sephadex G-75 column (40 mm \times 1500 mm). The fractions that contained uronic acid and were eluted in the void volume were pooled, dialysed against water and freeze-dried.

The purified proteoglycans were finally subfractionated by dissolving them (1–5 mg of proteoglycan mixture) in 5% (w/v) calcium acetate/0.5 M-acetic acid, pH 5.0, at 4 °C over a period of 24 h. The insoluble material was removed by centrifugation at 20000 *g* for 30 min at 4 °C. Cold ethanol was added dropwise to the clarified supernatant with vigorous stirring to give a final concentration of 25% (v/v). Precipitate was allowed to form over 12 h at 4 °C and was then collected by centrifugation for 20000 *g* for 30 min at 4 °C. Ethanol concentration of the supernatant was adjusted in stages to 35% and 50% (v/v), and each precipitate was collected by centrifugation, washed with the corresponding ethanol/water mixture, followed by solubilization and dialysis against water at 4 °C. The dialysis residues were freeze-dried and stored desiccated at 4 °C. The proteoglycan fractions recovered by this procedure are subsequently referred to as PG-25, PG-35 and PG-50. The numbers correspond to the percentage concentration (v/v) of ethanol at which the respective proteoglycans are precipitated.

Preparation of glycosaminoglycans

Proteoglycan samples (1 mg/ml) were digested with papain according to the method of Scott (1960), with minor modifications described by Kapoor *et al.* (1981). The oligosaccharide and glycosaminoglycans were isolated by ion-exchange chromatography on a column (7 mm \times 100 mm) of Dowex AG 2X-8 (400 mesh; Cl^- form) or DE-23 DEAE-cellulose, eluted stepwise with 0.02 M-HCl, 0.3 M-NaCl and 2 M-NaCl. The oligosaccharide and the glycan chains were obtained from the first and last fractions respectively. The 0.3 M-NaCl fraction was less than 10% of the starting material (by hexosamine analysis) and was not further analysed.

Uronic acid, neutral sugar and amino sugar composition of polysaccharide chains

Neutral sugars were determined by g.l.c., after methanolic-HCl hydrolysis of polysaccharide chains followed by formation of trimethylsilyl derivatives as described by Bhatti *et al.* (1970), on a series 104 gas chromatogram (Pye-Unicam, Cambridge, U.K.).

The hexuronic acid contents of the polysaccharide chains were determined by the method of Bitter & Muir (1962). Total hexosamines were determined either by a modification of the Elson–Morgan reaction (Gardell, 1958) or by hydrolysis of the polysaccharide chains in 4 M-HCl at 100 °C for 8 h in sealed ampoules under an atmosphere of N_2 , followed by analysis of the hydrolysate for amino sugars on a Locarte amino acid analyser consisting of a single-column elution system.

Light-scattering

The weight-average M_r (M_w) values of proteoglycans were determined by light-scattering experiments in 4 M-GdnHCl/50 mM-Tris/HCl buffer, pH 7.4.

All buffers for light-scattering were clarified as described by Sheehan *et al.* (1978). The subfractionated proteoglycans, after clarification by centrifugation at 20000 *g* for 1 h, were filtered through a Micropore membrane (0.45 μm pore size) before being loaded into cells.

The intensity of light-scattering at angles between 30° and 150° was determined with the Sofica model 42000 photo-goniodiffusometer (Société Française d'Instruments de Contrôle et d'Analyses), with an unpolarized light-source of 436 nm. The increments in refractive index of proteoglycan solutions (4–5 mg/ml) versus the buffer were determined with a Brice–Phoenix differential refractometer (Phoenix Division of VirTis, Gardiner, NY, U.S.A.). The data were evaluated as described by Tomimatsu *et al.* (1968), and expressed in the form of Zimm plots.

Sedimentation velocity

The heterogeneity of proteoglycan fractions was assessed by ultracentrifugation. All such experiments were carried out at 20 °C, and samples were centrifuged at 216000 *g* in an MSE Centriscan operated under standard conditions. The Svedberg coefficient was determined in 4 M-GdnHCl at pH 7.4. Five samples of proteoglycans from each fraction, PG-25, PG-35 and PG-50, were dissolved at various concentrations (1–5 mg/ml) in the above buffer and dialysed against the same buffer for 12 h before centrifugation.

Traces of the peaks of sedimenting macromolecules were taken in the visible schlieren mode at regular time intervals and plotted on a chart recorder. The sedimentation coefficient, *s*, was determined by plotting the logarithm of the distance of a peak from the centre of rotation against time. All values of *s* were expressed in the form s^0 , which is the value of the coefficient at infinite dilution.

Viscometry

The viscosities of all proteoglycans in 4 M-GdnHCl/0.5 M-sodium acetate buffer, pH 7.4, were determined as described by Fransson *et al.* (1979) by using a Couette viscometer (Ogston & Stanier, 1953), modified to the cone-and-plate principle (Mooney & Ewart, 1934).

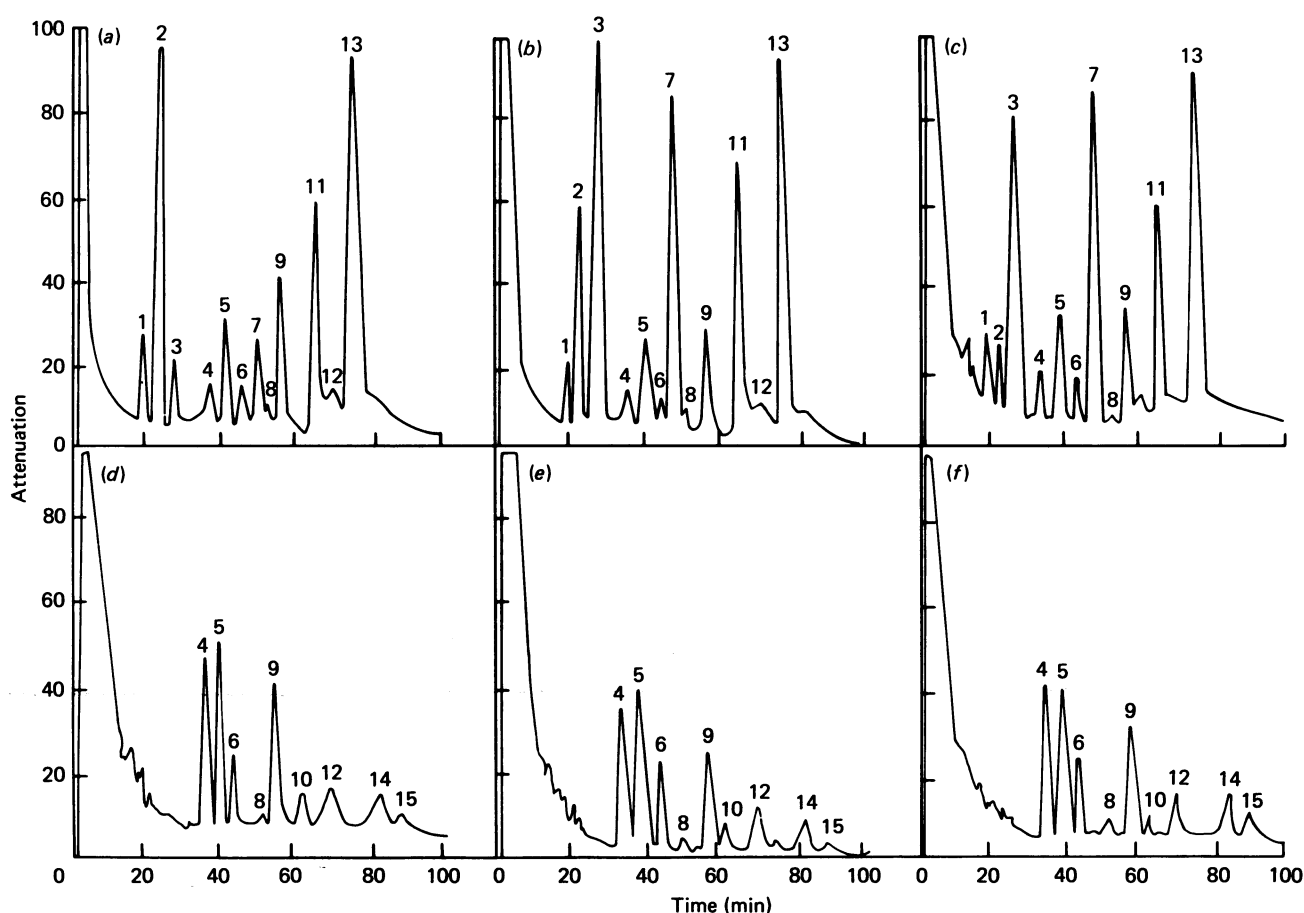


Fig. 1. G.l.c. of the sugar chains derived from bovine aortic proteoglycans by papain digestion and separation of the products by ion-exchange chromatography

(a), (b) and (c) Chains obtained from PG-25, PG-35 and PG-50 respectively and eluted with 2 M-NaCl from the ion-exchange column. (d), (e) and (f) Oligosaccharide chains obtained from PG-25, PG-35 and PG-50, respectively and eluted with 0.02 M-HCl from the ion-exchange column. Key: 1, xylose; 2, xylose+IdoA; 3, GlcA; 4 and 5, galactose+mannose; 6, galactose; 7, GlcA; 8, glucose; 9, mannitol (internal standard); 10, glucosamine; 11, galactosamine; 12, glucosamine; 13, galactosamine; 14 and 15, glucosamine.

Electron microscopy

Freeze-dried samples were dissolved in 1 ml of 1 M-ammonium acetate buffer, pH 5.0, to give a final concentration of 400 μ g of proteoglycan/ml. Each sample was diluted either 1:10 or 1:100 with the same buffer and then mixed 1:1 with 10 mM-Tris/HCl buffer (pH 8.5)/1 mM-EDTA. Spreading solutions of each sample were then prepared by mixing 200 μ l of the sample with 5 μ l of cytochrome *c* (horse heart, Sigma type III; 2.5 mg/ml). The resulting solution was placed on a wet glass slide and allowed to spread over a hypophase of 0.3 M-ammonium acetate buffer, pH 5.0. The proteoglycan/cytochrome *c* monolayer was picked up on 300-mesh Parlodian/carbon-coated grids and, without draining, each grid was stained by immersion in 50 μ M-uranyl acetate in 90% (v/v) ethanol for 30 s and then rinsed in 90% ethanol for 30 s. Grids were air-dried and rotary-shadowed with platinum/palladium at an angle of 10° and distance of 4 cm. Grids were examined with a JEOL 100 B electron microscope and photographed at a magnification of 25000. Magnifications were calibrated by using a Pelco magnification-calibration grid. Negatives were enlarged to give working prints at a final magnification of 66450.

Hyaluronic acid-proteoglycan binding

Hyaluronic acid was added (1%, w/w) to proteoglycans (3 mg/ml) in 0.5 M-sodium acetate buffer, pH 7.4. The mixture was kept at room temperature for at least 1 h. It was then analysed either by viscometry or by molecular-sieve chromatography on a Sepharose CL-4B column (0.7 cm \times 100 cm). The column was eluted with 0.5 M-sodium acetate buffer, pH 7.4, at a flow rate of 5 ml/h, and 0.5 ml fractions were collected and analysed for uronic acid by the method of Bitter & Muir (1962).

RESULTS

Uronic acid, neutral sugar and amino sugar composition

The carbohydrate chains obtained by papain digestion of different proteoglycan fractions were separated by ion-exchange chromatography into oligosaccharides (eluted with 0.02 M-HCl) and glycosaminoglycans (eluted with 2 M-NaCl). Analysis by g.l.c. indicated that oligosaccharides isolated from all proteoglycan fractions mainly contained galactose and glucosamine, with smaller amounts of mannose (Figs. 1d-1f). In contrast, the glycosaminoglycans consisted mostly of galactos-

Table 1. Chemical composition of chains derived from various proteoglycan fractions by papain digestion

The digested products were separated on an ion-exchange column eluted with a stepwise gradient (see the Experimental section). Trace, < 10 $\mu\text{mol/g}$ dry wt.; N.D., not detected.

	Composition ($\mu\text{mol/g}$ dry wt.)					
	0.02 M-HCl fraction			2 M-NaCl fraction		
	PG-25	PG-35	PG-50	PG-25	PG-35	PG-50
Glucosamine	98	210	251	Trace	Trace	Trace
Galactosamine	Trace	Trace	Trace	1491	1704	1500
Uronic acid	Nil	Nil	Nil	1300	1761	1700
Xylose	N.D.	N.D.	N.D.	21	24	25
Galactose	83	199	259	40	50	48
Mannose	32	27	53	N.D.	N.D.	N.D.

	Molar ratio					
	PG-25	PG-35	PG-50			
Xylose/galactose				1:1.90	1:2.08	1:1.92
Xylose/galactosamine				1:71	1:71	1:60
Galactose/glucosamine	1:1.07	1:1.05	0.97:1			
GlcA/IdoA*				1:3	7:1	100% GlcA
Total uronic acid/galactosamine				1:1.14	0.96:1	0.88:1

* Obtained from Kapoor *et al.* (1981).

Table 2. Solution properties of bovine aortic proteoglycans

All physical parameters of proteoglycan fractions were determined in 4 M-GdnHCl

	$10^{-6} \times M_w$	s^0 (S)	$[\eta]$ (ml/g)
PG-25	1.30	7	375
PG-35	0.30	7	152
PG-50	0.88	8	96

amine and large amounts of IdoA and GlcA (Figs. 1a–1c). The content of the uronic acids IdoA and GlcA was variable. PG-25 contained more IdoA than GlcA, whereas PG-35 and PG-50 were enriched with GlcA.

The estimates of neutral sugars by g.l.c. and of hexuronic acid and hexosamines by alternative methods (see the Experimental section) are shown in Table 1. All glycosaminoglycans (2 M-NaCl fractions) contained an approximately unitary hexuronic acid/hexosamine ratio, a xylose/galactose ratio of 1:2 and an absence of mannose. Glycosaminoglycans from fractions of PG-25 and PG-35 had a xylose/hexosamine ratio of approx. 1:70, whereas this ratio was slightly lower (1:60) for fraction PG-50. In contrast, the oligosaccharides (0.02 M-HCl fractions) contained no hexuronic acid and a galactose/hexosamine ratio of approx. 1:1 (Table 1). The nature of amino sugars present in oligosaccharide and glycosaminoglycans was analysed by hydrolysis of the polysaccharide chains, followed by analysis of the hydrolysate on a Locarte amino acid analyser (see the Experimental section). Glucosamine was the predominant amino sugar in the oligosaccharide fractions, and the glycosaminoglycans contained galactosamine as their major amino sugar, thus supporting the results of the sugar analysis by g.l.c. shown in Fig. 1.

Light-scattering

M_w values of the aortic proteoglycans were assessed by light-scattering techniques in 4 M-GdnHCl. The results are summarized in Table 2, and the respective Zimm plots are shown in Fig. 2. By this method the M_w values of PG-25, PG-35 and PG-50 were 1.3×10^6 , 0.30×10^6 and 0.88×10^6 respectively. The deviation from linearity of all the Zimm plots suggested that the proteoglycans were polydisperse. In particular, the angular dependence of scattering at constant concentration passed through a minimum for PG-35 (Fig. 2b), which may be due to the fluctuation in orientation of the molecules in solution resulting in optical anisotropy.

Sedimentation velocity

As the sedimentation coefficient is concentration-dependent, for bovine aortic proteoglycans the s value diminished with increasing polymer concentration (results not shown). The s^0 values of the various proteoglycans were distributed between 7 to 8 S, and did not vary in proportion with their M_w values (Table 2). The sedimentation profile of the proteoglycans was unimodal under associative and dissociative conditions (results not shown), except for PG-25, which showed a bimodal distribution under associative conditions (Fig. 3).

Viscometry

The viscosities of proteoglycan fractions at various concentrations and shear rates were determined in 4 M-GdnHCl/50 mM-Tris/HCl buffer, pH 7.4. The viscosities of all proteoglycans were concentration- and shear-dependent, although PG-35 and PG-50 showed pronounced shear-dependence only at lower concentrations (Fig. 4). The experimental data expressed in terms of the intrinsic viscosity $[\eta]$ at zero shear rate and infinite dilution of the proteoglycans is shown in Table 2. The large $[\eta]$ of the various proteoglycans is consistent with

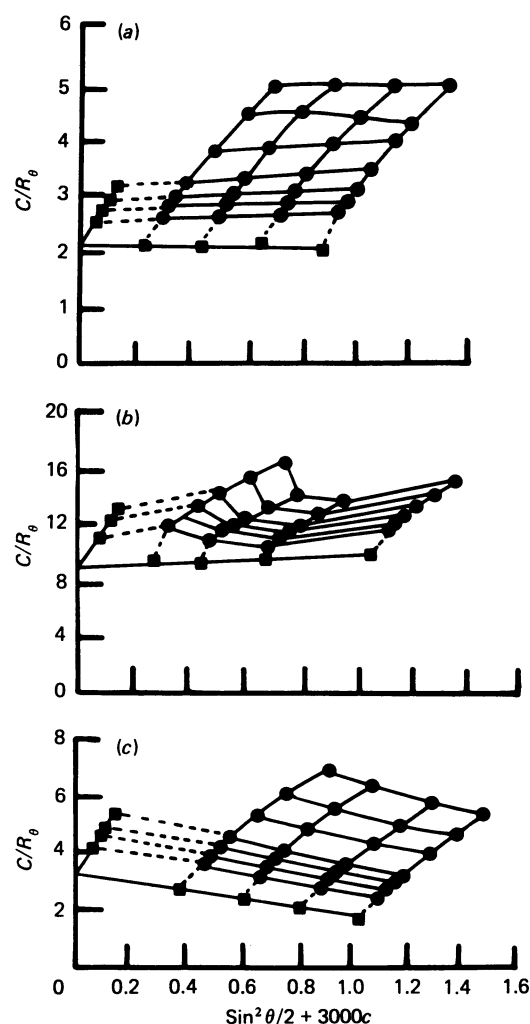


Fig. 2. Zimm plots for the light-scattering data of the chondroitin sulphate/dermatan sulphate proteoglycans

All experiments were carried out in 4 M-GdnHCl at pH 7.4 at 20 °C. (a) PG-25 at concentrations of 884, 663, 442 and 221 $\mu\text{g/ml}$; $\Delta n/\Delta c$ 0.140 ml/g. (b) PG-35 at concentrations of 1075, 860, 645 and 215 $\mu\text{g/ml}$; $\Delta n/\Delta c$ 0.136 ml/g. (c) PG-50 at concentrations of 349, 279, 209 and 139 $\mu\text{g/ml}$; $\Delta n/\Delta c$ 0.134 ml/g. ■---■, Extrapolated points; ●—●, experimentally determined points.

their elongated rod-like or large highly branched structures.

Electron microscopy

Preparations of PG-25 contained three categories of spread molecules according to shape, size and degree of complexity. The smallest structural unit is shown in Fig. 5(a). This group consisted of molecules with an apparent long axis of 90–100 nm with two or three side projections averaging 75 nm in length. This group comprised approx. 33% of the total number of spread PG-25 molecules ($n = 112$). Almost half of the molecules (approx. 47%) appeared to form self-aggregated profiles, as shown in Fig. 5(b). These aggregated profiles tended to be of uniform size, with a long axis of approx. 211 nm and a diameter axis of approx. 150 nm. Some of the spread PG-25 molecules (approx. 19%) appeared to form superaggregates, as demonstrated in Fig. 5(c). These

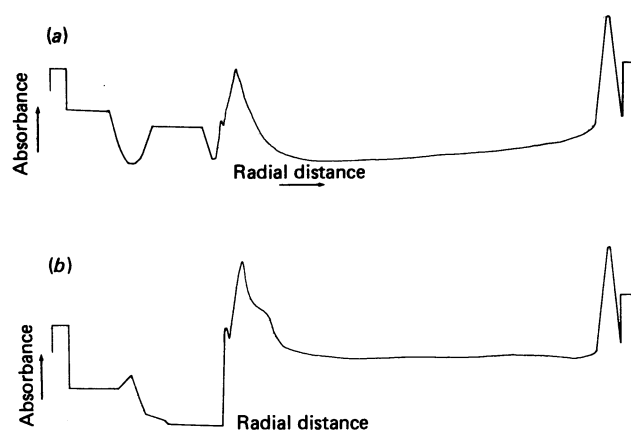


Fig. 3. Sedimentation profiles of PG-25 (3 mg/ml) in sedimentation-velocity experiments under associative and dissociative conditions

(a) PG-25 in 4 M-GdnHCl. (b) PG-25 in 0.15 M-NaCl.

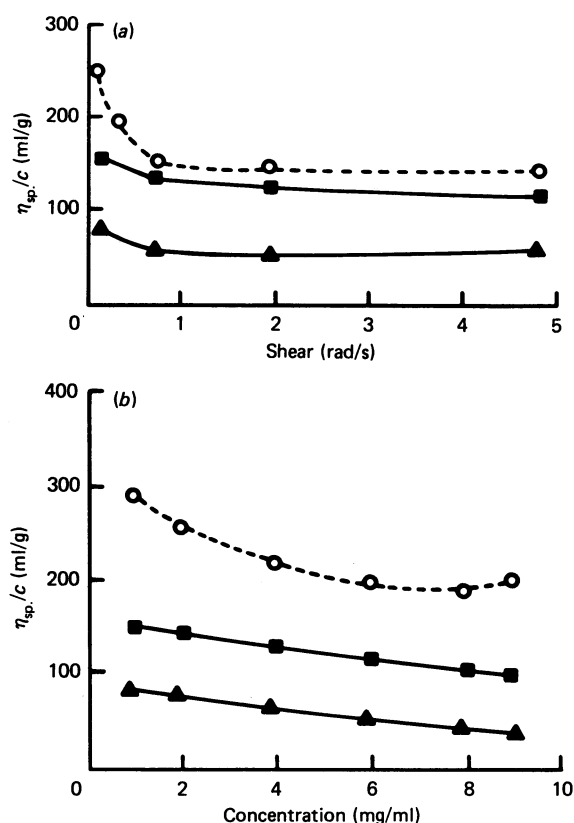


Fig. 4. Reduced viscosity of chondroitin sulphate/dermatan sulphate proteoglycans

The viscosity measurements were made at various proteoglycan concentrations and shear speeds in 4 M-GdnHCl at pH 7.4 at 10 °C. ○, PG-25; ■, PG-35; ▲, PG-50.

structures frequently appeared to consist of clusters of the profiles shown in Fig. 5(b).

PG-35 differed from PG-25 in that mainly single-monomer profiles were observed, with a minute proportion of the monomers forming small aggregates (Figs. 5d and 5e). The smallest unit identified in this preparation

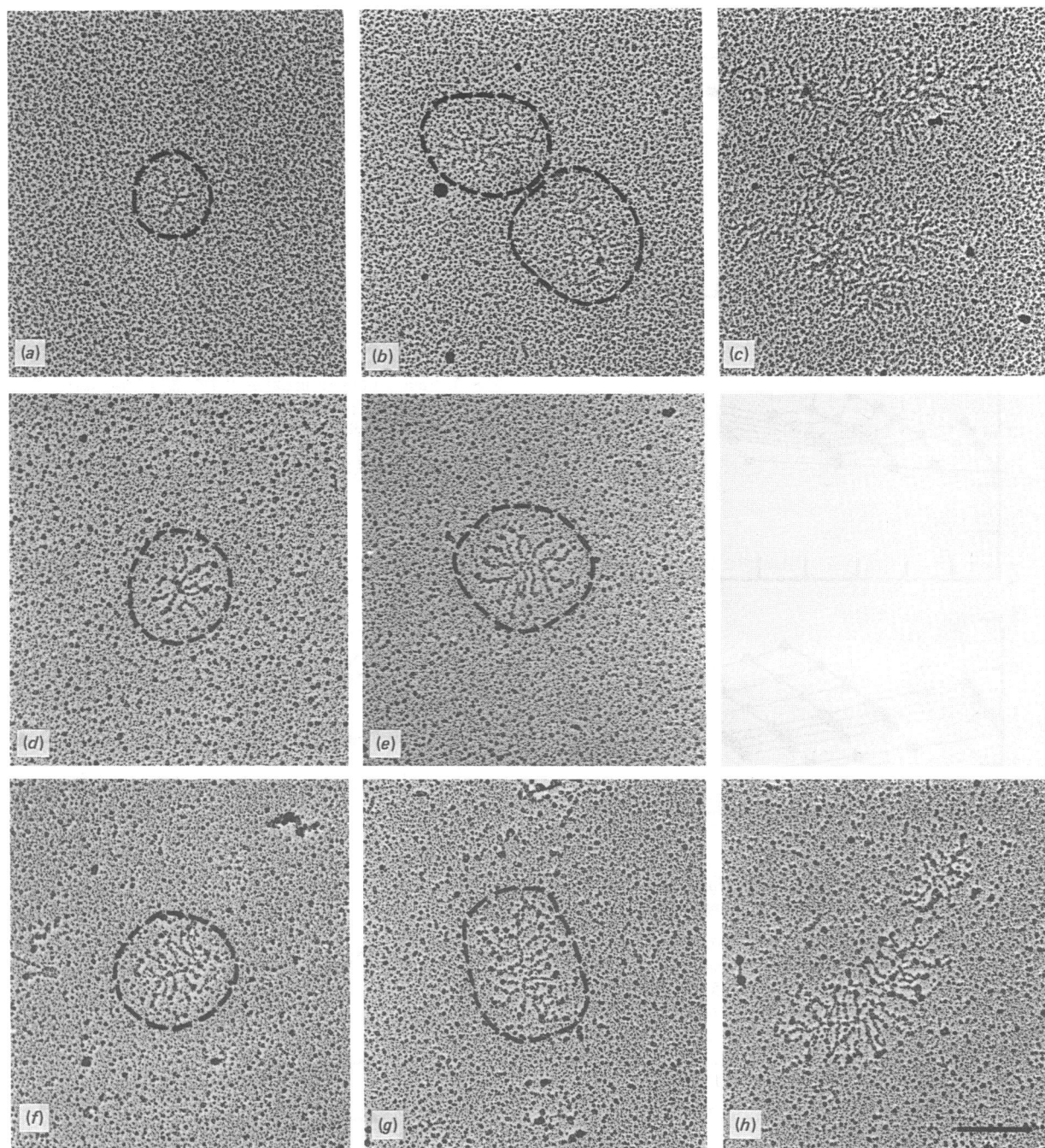


Fig. 5. Electron micrographs of vascular proteoglycans detected by the rotary-shadowing technique

(a)–(c) PG-25; (d) and (e) PG-35, (f)–(h) PG-50. Bar represents 0.25 μm for all micrographs.

was characterized by a long axis of approx. 75 nm to which were attached eight to ten side projections ranging in length from 90 nm to 135 nm (Fig. 5d). The more common profile (approx. 70% of total) consisted of a structure whose long axis was approx. 135 nm and contained approx. nine to 12 side projections of average length of 120 nm (Fig. 5e).

PG-50 spreads contained the largest monomer profiles and unlike PG-35 and PG-25 exhibited no evidence of self-aggregation (Figs. 5f–5h). The long axis of these molecules ranged from 150 nm to 240 nm, and each monomer contained between ten and 13 side projections. These side projections were not as long as those observed

in the PG-35 preparation and averaged approx. 90 nm. Occasionally two or more of these monomers appeared aligned end-to-end (Fig. 5h).

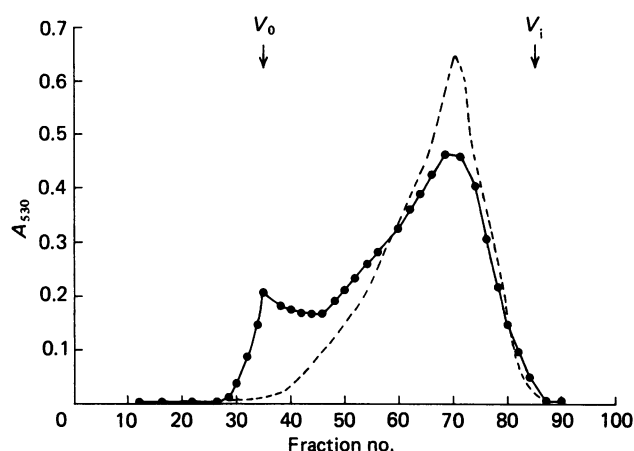
Hyaluronic acid binding to the proteoglycan fractions PG-25, PG-35 and PG-50

Changes in the reduced viscosity of proteoglycan solutions in the presence and in the absence of hyaluronic acid were used as a measure of the extent of hyaluronic acid binding to the proteoglycans. The reduced viscosity of PG-50 remained unchanged in the presence of hyaluronic acid. In contrast, the addition of hyaluronic acid to PG-35 caused a 10.7-fold increase in

Table 3. Reduced viscosity of bovine aortic proteoglycans in the presence and in the absence of 1% (w/w) hyaluronic acid

The reduced viscosity was determined at a proteoglycan concentration of 3 mg/ml in 0.5 M-sodium acetate buffer, pH 7.4.

		Reduced viscosity (ml/g)
PG-25	With hyaluronic acid	Indeterminable
	Without hyaluronic acid	Indeterminable
PG-35	With hyaluronic acid	1600
	With hyaluronic acid + 0.6% (w/w) oligosaccharides	180
	Without hyaluronic acid	150
PG-50	With hyaluronic acid	170
	Without hyaluronic acid	150

**Fig. 6. Molecular-sieve chromatography of PG-35 on Sepharose CL-4B**

The proteoglycans were chromatographed in the presence (●—●) and in the absence (----) of hyaluronic acid in 0.5 M-sodium acetate buffer, pH 7.4. The column was eluted at a flow rate of 5 ml/h, and 0.5 ml fractions were collected. The void volume (V_0) and the included volume (V_i) of the column were determined by eluting hyaluronic acid-proteoglycan complexes and Phenol Red respectively.

the reduced viscosity of this proteoglycan solution (Table 3). This increase in the viscosity of PG-35 was inhibited by the addition of oligosaccharides to the PG-35/hyaluronic acid mixture. The proportion of PG-35 molecules interacting with hyaluronic acid was further studied by molecular-sieve chromatography. The results indicated that only a small fraction of the molecules in PG-35 were capable of binding with hyaluronic acid (Fig. 6).

PG-25 formed a viscous solution, and reliable measurements for its reduced viscosity could not be obtained. Hyaluronic acid binding to PG-25 was

therefore studied by other techniques, such as molecular-sieve chromatography, equilibrium dialysis and ultracentrifugation. These analyses showed PG-25 did not bind to hyaluronic acid. Similarly, PG-50 also showed a lack of hyaluronic acid binding (results not shown).

DISCUSSION

Blood vessels contain a mixture of proteoglycans defined mainly by their hydrodynamic size and glycosaminoglycan composition. Studies in man (Salisbury & Wagner, 1981), pig (Breton *et al.*, 1981), pigeon (Wagner *et al.*, 1983; Rowe & Wagner, 1985) and bovine (Eisenstein *et al.*, 1975; Oegema *et al.*, 1979; Seethanathan *et al.*, 1980; Kapoor *et al.*, 1981; Schmidt *et al.*, 1982; Murray, 1983; Radhakrishnamurthy *et al.*, 1986) indicate that there are at least three families of arterial proteoglycans: chondroitin sulphate proteoglycans, dermatan sulphate proteoglycans and heparan sulphate proteoglycans. These families differ with respect to hydrodynamic size, molecular mass and ability to aggregate with hyaluronic acid. In the present study bovine aortic chondroitin sulphate and dermatan sulphate proteoglycans were characterized after their fractionation by selective ethanol precipitation, as previously described by Kapoor *et al.* (1981). Unlike earlier studies on aortic proteoglycans, which were fractionated according to the number of side chains on the core protein by density-gradient centrifugation (Oegema *et al.*, 1979; McMurtry *et al.*, 1979; Radhakrishnamurthy *et al.*, 1982; Wagner *et al.*, 1983), our procedure resulted in the separation of three proteoglycan fractions, PG-25, PG-35 and PG-50, according to the IdoA and GlcA contents of the cognate glycan chains. By this method, one pool of proteodermatan sulphate, PG-25, one pool of proteochondroitin and dermatan sulphate, PG-35 (major fraction), and a separate pool of proteochondroitin sulphate, PG-50, were obtained, representing 28%, 45% and 27% respectively of the total extracted material. Subjecting the different proteoglycan fractions to detailed chemical analysis revealed the following. (1) A fraction of the molecules in PG-25 self-associated to form superaggregates. PG-35 consisted mainly of proteoglycan monomers, a very small percentage of which associated to larger aggregates, and PG-50 showed no tendency to self-associate. It is noteworthy that PG-25 is dermatan sulphate proteoglycan-like and previous studies have reported that dermatan sulphate chains can self-associate to form superaggregates (Fransson *et al.*, 1979; Rosenberg *et al.*, 1985). It is possible that the high estimate of the M_w of PG-25 (i.e. 1.3×10^6) observed in the present study was due to the tendency of these monomers to self-associate and form aggregates of definitive size, which do not fully dissociate when M_w estimates are done in 4 M-GdnHCl. This finding is further supported by the studies by Salisbury & Wagner (1981) and Schmidt *et al.* (1982), which showed that aggregates of aortic proteoglycans may not fully dissociate in 4 M-GdnHCl. (2) The Zimm plots for the proteoglycans obtained by light-scattering deviated from linearity, suggesting polydispersity of molecular species in the different proteoglycan fractions. This observation was consistent with their broad distribution on molecular-sieve chromatography (results not shown), which also indicated some polydispersity of

size in each fraction. The M_w determined by light-scattering for each proteoglycan fraction are therefore weight-average. (3) The s^0 values of all proteoglycans were very similar and did not reflect differences in their molecular masses. This is probably because with entanglements of elongated and/or branched structures s^0 is no longer a measure of the molecular mass (Kitchen & Cleland, 1978). (4) The high $[\eta]$ of the different proteoglycan fractions reflected their highly branched or elongated rod-like structures. The very large $[\eta]$ of PG-25 was consistent with its tendency to form superaggregates. The high $[\eta]$ of PG-35 relative to its low M_w could be due partly to the tendency of a fraction of these molecules to form small aggregates. (5) Previous studies have shown that a fraction of aortic proteoglycans bind to hyaluronic acid (Oegema *et al.*, 1979; McMurtry *et al.*, 1979; Salisbury & Wagner, 1981; Wagner *et al.*, 1983). Our results specifically indicated that only a small proportion of PG-35 complexed with hyaluronic acid. The interaction of PG-35 with hyaluronic acid could be inhibited by oligosaccharides, suggesting similarities with hyaluronic binding to cartilage proteoglycans, which is also inhibited by oligosaccharides (Hardingham & Muir, 1973; Hascall & Heinegård, 1974). It is unlikely that the fraction of molecules in PG-35 interacting with hyaluronic acid represents a contaminating molecular species from PG-25 or PG-50. This is based on the fact that these latter fractions did not show hyaluronic acid-binding property by several different experimental criteria.

Papain digestion of the proteoglycans resulted in the isolation of two fractions, oligosaccharides and glycosaminoglycans. The oligosaccharide fraction was eluted at low ionic strength on an ion-exchange column, contained no uronic acid, had a small amount of mannose and a unitary galactose/glucosamine ratio. This was suggestive of keratan sulphate-like chains (Greiling *et al.*, 1970; Stuhlsatz *et al.*, 1971; Handley & Phelps, 1972; Hopwood & Robinson, 1974a,b; Nilsson *et al.*, 1983; Kapoor & Prehm, 1983), although other *N*- and *O*-linked oligosaccharides cannot be ruled out (Gowda *et al.*, 1986a,b). It is unlikely that the oligosaccharides represent a separate glycoprotein-like molecular species, as they were obtained after papain digestion of proteoglycan fractions, which were extensively purified by several techniques that did not favour the co-purification of glycoproteins. It is therefore possible that the oligosaccharide chains are linked to the same core protein as the glycosaminoglycan chains. This would be consistent with the previous observation on bovine aortic (Oegema *et al.*, 1979) and cartilage proteoglycans (Heinegård & Axelsson, 1977; Ratcliffe *et al.*, 1985), which have been shown to contain oligosaccharide and glycosaminoglycan chains linked to the same core protein.

The glycosaminoglycan chains consisted of uronic acid, a xylose/galactose ratio of 1:2, and galactosamine as the predominant amino sugar (Table 1). Such glycosaminoglycan chains were chemically similar to chondroitin sulphate or dermatan sulphate. On the basis of the molecular mass of the proteoglycans and their molar ratio of xylose to galactosamine, an average of 27 chains each consisting of approx. 70 disaccharide units can be calculated for PG-25. However, it should be emphasized that these proteoglycans have a tendency to form aggregates and therefore these estimates might reflect the average number of chains in an aggregate

rather than representing the monomeric form. Similarly, PG-35 consisted of an average of seven chains each of approx. 70 disaccharide units. In contrast, PG-50, which did not exhibit a tendency to self-associate, consisted of an average of 22 chains each of approx. 60 disaccharide units. These estimates were further supported by morphological observations of the individual preparations. For example, the smallest unit within PG-25 consisted of a core ranging in length from 90 to 100 nm to which were attached two or three side projections. If it is assumed that the long axis of this structure represents the protein moiety of the proteoglycan, an M_r estimate can be calculated to be approx. 45000–50000 on the basis of the assumption that an amino acid is 0.2 nm in length and has an M_r of 100 (Hascall, 1980). This is within the size range of other IdoA-rich dermatan sulphate core proteins identified in a number of other tissues (Pearson & Gibson, 1982; Damle *et al.*, 1982; Hassell *et al.*, 1984; Glossl *et al.*, 1984; Vogel *et al.*, 1984; Rosenberg *et al.*, 1985), including blood vessels (Rowe & Wagner, 1985). In addition, electron microscopy of a small dermatan sulphate proteoglycan isolated from cultures of arterial smooth-muscle cells reveals a similar structure to those monomers present in PG-25 (Wight, 1980). The length of the majority of the side projections associated with this core measured approx. 75 nm. If these side projections represent glycosaminoglycan chains, it can be estimated that the chains contain about 75 disaccharides since the length of a disaccharide is about 1 nm (Hascall, 1980). This value is close to the value (70) obtained by chemical analysis of PG-25 to estimate the number of disaccharides present in each glycosaminoglycan side chain linked to the core protein of PG-25.

The presence of a mixture of IdoA and GlcA in PG-25 and PG-35 may be associated with co-polymericity of the glycan chains, as previously suggested for aortic proteoglycans by Radhakrishnamurthy *et al.* (1982, 1986). The extent to which this chemical and physical heterogeneity of aortic proteoglycans affects the properties of the tissue is not completely understood. It has been suggested that structural differences in the proteoglycans, together with their unequal distribution in the arterial wall (Wight & Ross, 1975; Wight, 1980), lead to variable interactions between these and other extracellular matrix components and that these may modulate the properties of connective tissues (Phelps, 1975). In this context, it is noteworthy that a combination of the co-polymeric nature, self-association and hyaluronate-dependent aggregation of proteoglycans could endow the aortic tissue matrix with a marked resistance towards compression and deformation.

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